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SHOOT AND ROOT FORMATION OBTAINED ON SELECTED SPECIES OF THE GESNERIACEAE THROUGH TISSUE-CULTURE

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Growth responses of leaf explants in sterile culture were studied for ten taxa chosen from both subfamilies to represent the wide structural diversity of the Gesneriaceae: *Chirita sinensis* Lindl., *Streptocarpus saxorum* Engl., *Streptocarpus* 'Constant Nymph,' *Saintpaulia ionantha* Wendland, in the Cyrtandroideae; and *Koellikeria erinoides* (DC.) Mansf., × *Koellikohleria rosea* Wiehl., *Nematanthus perianthomegus* (Vell.) H. E. Moore, *Sinningia cardinalis* (Lehm.) H. E. Moore, *Sinningia speciosa* (Lodd.) Hiern, and *Sinningia tubiflora* (Hook.) Fritsch in the Gesnerioideae. An array of 26 combinations of kinetin, indoleacetic acid, naphthaleneacetic acis, and coconut milk in agar media was used; plant responses in terms of shoot, root, and callus production were recorded. Initiation of numerous vigorous shoots occurred in *Chirita sinensis*, *Streptocarpus* 'Constant Nymph,' *Koellikeria erinoides*, and *Sinningia speciosa*. After subculture on rooting medium, plants of these four taxa were potted and grown to maturity. Shoot initiation also occurred in *Streptocarpus saxorum* and *Saintpaulia ionantha* but the shoots were few and poor in vigor. Implications of the results for tissue-culture propagation of Gesneriaceae are discussed as possible correlations between culture responses and taxonomic relationships within the family.

INTRODUCTION

Tissue-culture techniques for propagation of the gesneriad *Sinningia speciosa* (Florist-Gloxinia) utilizing shoot apices (Haramaki, 1971) and peduncles (Bigot, 1974) have been reported. However, these techniques entail disadvantages: apex culture has limited value for single-crowned species and requires delicate surgical manipulations; and peduncle culture is restricted to times when the plant is in flower and prevents use of the parent for hybridizing. On the other hand, the use of leaves as a source of culture tissue offers several advantages, i.e., simplicity of culture preparation, continuous availability of source matierial, and avoidance of destruction of the parent plant.

The Gesneriaceae contain about 125 genera with over 2,000 species in two subfamilies; about 300 of the species are currently in cultivation (Skog, 1976). This report presents results of a study of 10 taxa of the family; the investigation was designed to examine their potentialities for shoot formation in leaf-explant culture on selected media and to ascertain the suitability of that culture technique for their clonal propagation.

MATERIALS AND METHODS

Species and hybrids were selected to represent the two subfamilies, Cyrtandroideae and Gesnerioideae, of the Gesneriaceae (Fritsch, 1893, 1894), as well as the wide morphological diversity of the family. The taxa selected were *Chirita sinensis* Lindl., *Streptocarpus saxorum* Engl., *Streptocarpus* 'Contant Nymph,' *Saintpaulia ionantha* Wendland, in the Cyrtandroideae; and *Koellikeria erinoides* (DC) Mansf., × *Koellikohleria rosea* Wiehl., *Nematanthus perianthomegus* (Vell.) H. E. Moore, *Sinningia cardinalis* (Lehm) H. E.

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Moore, *Sinningia speciosa* (Lodd.) Hiern, and *Sinningia tubiflora* (Hook.) Fritsch, in the Gesnerioideae.

The study consists of a series of 30 preliminary experiments, involving 10 taxa (above), to investigate the responses to a variety of growth factors for shoot and root formation on leaf, stem, and peduncle explants, and a confirmation experiment involving leaf explants of 8 taxa on an array of 26 growth factor concentrations (Table 1). Each of the preliminary experiments was repeated at least once. The confirmation experiment, which was repeated once, was based on an evaluation of the preliminary experiments. In all of the experiments, the treatments of each taxa were replicated by five explants contained singly in separate bottles.

For the duplicate confirmation experiments, mature leaves were taken from plants maintained in the greenhouse and surface-sterilized by a procedure which utilized 20% H_2O_2 for 5 minutes and a subsequent antioxidant rinse (1.0 mM citric acid and 1.0 mM L-ascorbic acid) (Haramaki, 1971). Centimeter-square pieces were cut from the blades except in the case of *Streptocarpus saxorum*, where whole leaves were used. The explants were placed abaxial side down in the culture bottles, which were laid on their sides at 23-27°C under continuous cool-white fluorescent light of approximately 2850 lux. Each experimental treatment was replicated by five explants contained singly in separate bottles.

The culture medium, which was solidified with 1% agar, was basically that of Murashige and Skoog (1962), but with thiamine(0.4 mg/1) as the only vitamin supplement. Growth factors added were kinetin (0.5-10.0 mg/1) plus an auxin, either indoleacetic acid (0.1-5.0 mg/1) or naphthaleneacetic acid (0.1-5.0 mg/1). Coconut milk at 10% was also used without additional growth factors. The medium was melted, poured into 60-ml glass screw-cap bottles at the rate of 10 ml per bottle, and autoclaved at 2 atm for 15 minutes.

Explants which developed vigorous shoots were transferred to a rooting medium which was the same as that used for the initial cultures, but without kinetin and with increased auxin levels. The subcultures were maintained under the same light and temperature regime as the initial cultures but in larger bottles (placed on their sides), each containing 20 ml of medium. This rooting procedure was repeated at least four times for each taxon. Rooted shoots were separated, potted, and grown to maturity under constant cool-white fluorescent light at 27°C.

EXPERIMENTAL RESULTS

The preliminary experiments involved 10 taxa including *Saintpaulia ionantha* (Cyrtandroideae) and × *Koellikohleria rosea* (Gesnerioideae). Shoots formed over the entire adaxial and abaxial surface of the *Saintpaulia ionantha* explants and the veins of the abaxial surface only of the × *Koellikohleria rosea* explants. However, space dictated that the confirmation experiments be limited to 8 taxa so these 2 species were not included in the final experiments.

The 8 taxa involved in the confirmation experiment and its duplicate consisted of *Chirita sinensis*, *Streptocarpus saxorum*, *Streptocarpus* 'Constant Nymph' (Cyrtandroideae); and *Sinningia cardinalis*, *Sinningia tubiflora*, *Sinningia speciosa*, *Koellikeria erinoidea*, and *Nematanthus perianthomegus* (Gesnerioideae). The confirmation experiments consisted of two parts, the 8 week primary culture of the explants, (the results of which are tabulated in Table 1), and the 30-40 day subculture of the shoots (the results of which are tabulated in Table 2).

Table 1. Growth responses of leaf explants of eight gesneriads after eight weeks in culture on various growth-factor combinations.

K mg/l	Auxin mg/l	Subfamily Cyrtandroideae			Subfamily Gesnerioideae				
		Chirita sinensis	Streptocarpus saxorum	Streptocarpus 'Constant Nymph'	Koellikeria erinoides	Nematanthus perianthomegus	Sinningia cardinalis	Sinningia speciosa	Sinningia tubiflora
0	IAA								
	0	S^1R^1	N	S^1R^1	X	N	N	N	N
	0.1	S^2R^1	N	S^1R^2	X	N	N	R^1	N
	1.0	S^3 C^2	N	N	N	N	X	$S^2R^2C^1$	N
	5.0	S^1R^1	N	N	R^1	N	N	R^1	N
	NAA								
	0.1	S^2	R^1	S^2R^2	N	N	X	S^3*R^2*	N
	1.0	S^1R^1	N	R^1	N	N	X	R^2*C^1	N
	5.0	N	N	R^2	N	N	X	X	N
0.5	IAA								
	0	S^1	C^1	S^1	N	N	X	S^3 C^1	N
	0.1	S^2	N	S^1	N	N	X	N	N
	1.0	S^2	N	S^3R^2	N	N	X	R^1	N
	5.0	S^2	S^2 C^1	S^1	R^1	C^1	C^1	X	X
	NAA								
	0.1	S^2	N	R^2	N	C^1	X	X	N
	1.0	S^3	R^1	N	N	C^1	X	C^1	N
	5.0	$S^2R^1C^1$	N	N	N	R^1C^2	C^2	N	N
3.0	IAA								
	0	S^2	S^2 C^1	S^1	N	N	X	X	N
	0.1	S^1	S^2 C^1	S^1	N	N	X	X	N
	1.0	S^3*	C^1	S^3R^1	N	N	X	X	N
	5.0	S^2	C^2	S^2	X	C^1	X	X	X
	NAA								
	0.1	S^2	C^2	S^3*R^2	$S^2R^1C^1$	N	X	$S^3*R^1C^1$	N
	1.0	S^1	$S^1R^1C^2$	S^2R^1	S^1	C^1	X	$S^1R^1C^1$	C^1
	5.0	S^1R^1	N	N	X	X	N	R^2C^2	X
10.0	IAA								
	0.1	S^1	N	S^1	N	N	X	N	X
	5.0	S^1	N	$S^1R^1C^1$	C^1	N	C^1	N	N
	NAA								
	0.1	S^1	$S^1R^1C^2$	$S^1R^2C^2$	N	N	C^2	N	N
	1.0	S^1 C^2	R^2C^2	N	C^1	N	R^1C^2	R^2C^2	N
0	CM 10%	S^1R^1	N	S^1R^1	N	X	X	N	N

Taxon	Auxin 0 mg/1	IAA 1.0 mg/1	IAA 10 mg/1	NAA 1.0 mg/1
Root initiation				
Chirita sinensis	P	G	M	G
Streptocarpus 'Constant Nymph'	P	G	N	S
Sinningia speciosa	X	N	X	G
Koellikeria erinoides	X	N	X	G
Shoot regeneration				
Chirita sinensis	N	M	G	N
Streptocarpus 'Constant Nymph'	N	P	G	S
Sinningia speciosa	X	N	X	N
Koellikeria erinoides	X	N	X	G

KEY: G = Good P = Poor to none
M = Medium S = Shoots died
N = None X = Not done

Table 2. Responses of tissue-culture-initiated shoots on subculture media lacking kinetin but with different auxin complements.

Under some treatments, vigorous and prolific shoot production occurred in *Chirita sinensis*, *Streptocarpus* 'Constant Nymph', and *Sinningia speciosa*, and poor shoot production occurred in *Streptocarpus saxorum* and *Koellikeria erinoides*. No shoots were formed by *Sinningia tubiflora*, *Sinningia cardinalis*, or *Nematanthus perianthomegus* under any of the treatments.

In *Chirita sinensis*, *Streptocarpus saxorum*, and *Streptocarpus* 'Constant Nymph' (Cyrtandroideae), shoots formed over the entire adaxial and abaxial surfaces of the explants, whereas in *Sinningia speciosa* and *Koellikeria erinoides* (Gesnerioideae), the shoots developed only on the veins of the abaxial surface of the explants.

The best medium for shoot production on an overall family basis contained kinetin at 3.0 mg/1. At this kinetin level, *Chirita sinensis* and *Streptocarpus saxorum* gave best results with indoleacetic acid at 1.0 mg/1; *Sinningia speciosa* and *Koellikeria erinoides*, on the other hand, gave better results with naphthaleneacetic acid. *Streptocarpus* 'Constant Nymph' responded about equally to indoleacetic acid at 1.0 mg/1 and naphthaleneacetic acid at 0.1 mg/1, the difference being that the shoots were more vigorous with the latter. None of the taxa produced vigorous green shoots at the highest level (5.0 mg/1) of either auxin.

Roots were formed by at least one treatment on all taxa except *Sinningia tubiflora*. The number and vigor of the roots varied from few and nonprogressive to prolific and vigorous. In most cases the roots were either associated with shoots or initiated from the edges of the explants, but with *Streptocarpus* 'Constant Nymph' short fuzzy roots densely covered the entire adaxial surfaces of some explants on three of the media (kinetin 0 mg/1 + naphthaleneacetic acid 1.0 or 5.0 mg/1, and kinetin 3.0 mg/1 + naphthaleneacetic acid 1.0 mg/1). The entire adaxial surfaces of the *Streptocarpus saxorum* leaves were also covered with roots on one medium (kinetin 10.0 mg/1 + naphthaleneacetic acid 1.0 mg/1), but these roots were "normal" and continued to elongate with age.

Callus was formed in some treatments by most of the taxa, but it never became vigorous and dominant. Occasionally roots or shoots could be seen associated with this callus, but without exception the shoots referred to in Table 1 grew from the surfaces of the original explants. Sometimes callus was formed at the edges and on the veins of the explants several weeks after shoots were initiated from those explants, and occasionally additional shoots were produced in association with that callus. However, such additional shoot production was slow, inconsistent, and quantitatively small. This response is similar to that reported for Gerbera daisies by Murashige (1974), who found that large numbers of shoots were consistently produced from the original explant pieces, whereas shoots produced from callus were both few and inconsistent.

When vigorous shoots formed by *Chirita sinensis*, *Streptocarpus* 'Constant nymph', *Sinningia speciosa* and *Koellikeria erinoides* had produced at least two well-formed leaves, they were separated from the explants and transferred to subculture media formulated to enhance root development. Results after a subculture period of 30-40 days are summarized in Table 2. The response was either root production or shoot regeneration (continued shoot production). On a medium lacking auxin, rooting of both *Chirita* and *Streptocarpus* varied from poor to none. *Streptocarpus* rooted only on indoleacetic acid at 1.0 mg/1, but *Chirita* rooted well on lower levels of either auxin.

Sinningia and *Koellikeria* did not show shoot regeneration on any of the treatments, while *Chirita* and *Streptocarpus* produced moderate amounts of poor quality shoots on indoleacetic acid at 1.0 mg/1. The best responses for both *Chirita* and *Streptocarpus* were on indoleacetic acid at 10.0 mg/1, both in percentages of cultures that responded and in the numbers of shoots produced. Shoots which simply initiated roots grew rapidly and vigorously; after being transferred to pots, they reached maturity and flowered. The mature plants of all four taxa appeared to be identical to the parent stocks from which the explants had been taken.

The study results are somewhat complicated by severe contamination of the initial cultures by microorganisms in the cases of some taxa and by death of the explants in others. This kind of difficulty was also encountered by Murashige (1974), who obtained at best about 20% sterile, undamaged cultures with Gerbera daisies. There seemed to be a trend of difficulty with contamination from *Chirita sinensis* in which only 1.5% of the total number of cultures were contaminated, to *Sinningia cardinalis* in which 91% of the cultures were contaminated. Although contamination was not evident in most cultures of *Sinningia tubiflora*, the explants bleached and died within two weeks. Most cultures of *Nematanthus perianthomegus* remained alive and green for prolonged periods.

DISCUSSION

Although firm statements of medium requirements are not possible for all taxa studied, production of shoots by 5 of the 8 taxa studied suggests that all members of the Gesneriaceae may be capable of plantlet regeneration from leaf pieces in culture, provided appropriate media can be devised. The process of individual shoot formation was apparently similar in each of the cases observed, and most of the taxa showed at least promising responses to the growth factors used in the ranges of concentration tested. *Chirita sinensis* and *Streptocarpus* 'Constant Nymph' responded best and did so on a wide variety of growth factor combinations, producing literally hundreds of shoots

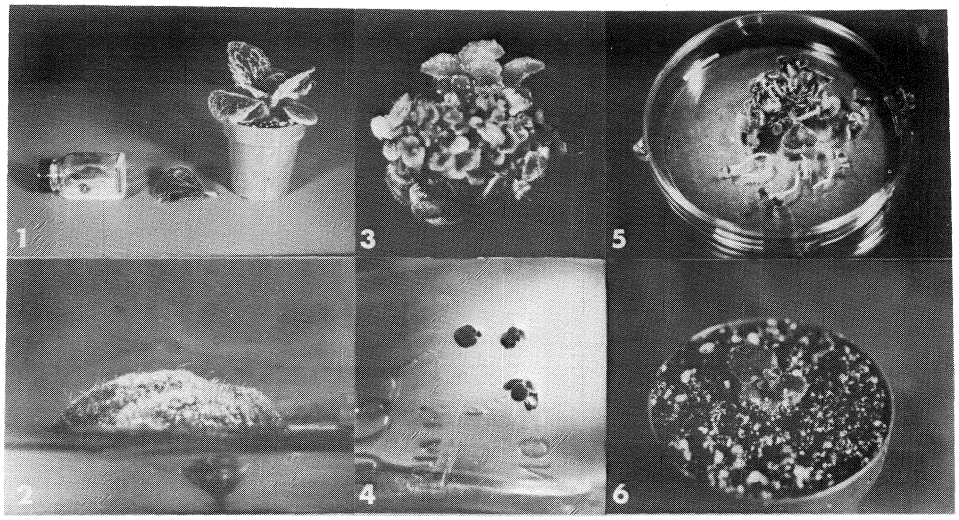

Fig. 1. *Chirita sinensis*, new explant in bottle of initial medium, plus leaf and suitable plant for explant material.

Fig. 2. *Chirita sinensis*, explant after 3 weeks in initial culture medium covered with emerging primordia.

Fig. 3 & 5. Explants with shoots of *Chirita sinensis* (3) and *Streptocarpus* 'Constant Nymph' (5) removed from initial culture medium fof division and transfer of shoots to subculture medium for rooting.

Fig. 4. Shoots of *Chirita sinensis* on subculture medium for root initiation.

Fig. 6. Potted young plant of *Chirita sinensis* 10 weeks from start of explant on initial medium.

per explant. *Sinningia speciosa* and *Koellikeria erinoides* produced shoots in smaller numbers, three to nine per explant.. The implications of these results for the propagation and multiplication of these gesneriads are apparent: in the cases of *Chirita* and *Streptocarpus*, tissue culture could provide an extremely large number of plants from one parent in a short time. Even in the case of *Sinningia speciosa* the advantage is clear: traditional leaf cuttings usually result in two or three plants per leaf; whereas a mature leaf under the conditions described would provide dozens of explants, many of which would produce shoots.

While broad conclusions regarding possible correlations between tissue-culture responses and taxonomic relationships within the family cannot be drawn from a study of such a small array of taxa, some of the results are suggestive. At least for the taxa, media, and conditions used here the members of the subfamily Cyrtandroideae showed a much stronger tendency toward organogenesis in culture than did members of the subfamily Gesnerioideae, Also, members of the former showed a pattern of shoot formation (over the entire explant surfaces) different from that of members of the latter (only on the veins of the abaxial surfaces). These tentative possibilities suggest that further work should be done with additional taxa, more media, other conditions, and different sterilization procedures to ascertain whether the responses observed are indeed related in some predictable way to taxonomic diversity within the family.

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LITERATURE CITED

Bigot, C. 1974. Obtention de plantes entières a partir de pédoncules floraux de Gloxinia hybrida cultivés in vitro. *Z. Pflanzenphysiol.* 73: 178-183.

Fritsch, K. 1893, 1894. Gesneriaceae. In: Engler, H. G. A., and Prantl, K. A. E. (eds.), *Die Naturlichen Pflanzenfamilien* 4 (3b): 133-144 (1893); 145-185 (1894).

Haramaki, O. 1971. Clonal propagation of orchids from leaf tips. *Amer. Orch. Soc. Bull.* 40: 109-113.

Murashige, T. 1974. Clonal multiplication of gerbera through tissue culture. *Hortscience* 9: 175-180.

Murashige T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant* 15: 473-497.

Skog, L. E. 1976. A study of the tribe Gesnerieae, with a revision of *Gesneria* (Gesneriaceae: Gesnerioideae). *Smithsonian Contributions to Botany,* Number 29.